REMARKS/ARGUMENTS

Claims 1-3, 10, 12-19, and 40-50 remain pending in this application, and are rejected. Claims 4-9, 11, and 20-39 have been withdrawn from consideration.

A. Claim Rejections Under Section 102

1. Gorovits Reference

In paragraphs 4-5 of the Office Action, the Examiner rejected Claims 1-3, 10, and 15-16 as being anticipated under 35 U.S.C. § 102 by the Gorovits Article (1997). Applicant respectfully traverses the rejection in light of the amended claims.

The present invention is directed to a novel method for folding a denatured polypeptide that involves two steps. First, the claims require that the polypeptide be bound to the chaperonin to form a chaperonin-complex. Second, the claims require "adding an osmolyte to the chaperonin-polypeptide complex, thereby promoting the folding of said polypeptide from its unfolded state to its folded state to yield a folded biologically active polypeptide." Since the osmolyte is added to the already-formed chaperonin-polypeptide complex, the chaperonin must first be added to the polypeptide to form a complex. Then, the osmolyte is added to the solution.

With this amended claim language, Applicant is expressly excluding instances in which the chaperonin-polypeptide mixture is merely "exposed" to the osmolyte by residual osmolyte being present in the mixture. Instead, the claims require the "addition" or active introduction of the osmolyte to the already-formed chaperonin-polypeptide complex.

The Gorovits Article does not teach or suggest the claimed invention. In the Gorovits Article, the urea was used to denature the DHFR protein itself (equivalent to step (a) of Applicant's claimed invention) -- not to promote the folding of the polypeptide as required by the claimed invention. After denaturing/unfolding the protein, the Gorovits Article indicates that the

unfolded protein was diluted with a buffer solution containing the chaperonin (GroEL) so that the urea concentration did not exceed 0.5M. Clearly, the urea (the purported osmolyte) was not "added" after the addition of the chaperonin. As such, there was no opportunity for the chaperonin-polypeptide complex to form prior to the addition of the osmolyte. Because the Gorovits Article does not teach or suggest addition of the osmolyte to the already-formed chaperonin-polypeptide complex, Applicant respectfully submits that the claimed invention is patentable in view of the Gorovits Article.

In contrast to the prior art, the precise order of this chaperonin/osmolyte protein folding process of the present invention is crucial to ensure successful folding of the protein in many instances. The folding protein is initially captured by the oligomeric chaperonin to form an arrested chaperonin-protein substrate complex. Applicant has repeatedly shown that this arrested form can hold to protein in a metastable but eventually foldable state for a long period of time. Once this complex is formed, the osmolyte solution(s) can then be added to the arrested chaperonin-protein substrate complex and released into the osmolyte solution where the ability of the osmolyte to influence successful protein folding occurs. The order of addition is crucial because there are numerous instances where osmolyte addition to a folding substrate alone prior to forming the arrested chaperonin-protein complex results in large scale protein misfolding (Voziyan et al., 2000; Voziyan and Fisher, 2000, attached to November 24, 2003 IDS).

In short, the Gorovits Article does not teach or suggest first forming a chaperonin-osmolyte complex and then "adding the osmolyte to the chaperonin-polypeptide complex" as required by the claims. Thus, Applicant requests that the Examiner withdraw the rejection under Section 102.

In addition, as discussed previously, the Gorovits Article does not teach or suggest urea (the purported osmolyte) to promote folding as required by the claimed invention. The folding of the protein DHFR was initiated in the presence of GroEL with residual urea present. However, the inclusion of urea did not in any way ***promote*** the folding of DHFR to its native conformation as required by the claimed invention. Further, there is not any teaching or suggestion that the resulting "promot[ion] is greater than that which is achieved using chaperonins and osmolytes alone". As such, for this additional reason, Applicant requests that the Examiner withdraw the rejection under Section 102.

2. Altamirano References

In paragraphs 6-7 of the Office Action, the Examiner rejected Claims 1-3, 10, 13-16 as being anticipated under 35 U.S.C. § 102 by Altamirano (1997) or Altamirano (1999) (together the Altamirano Articles). As discussed above, the claimed invention requires that the chaperonin-protein complex must be formed prior to the "addition" of the osmolyte. Because the Altamirano Articles do not teach or suggest such a step, Applicant respectfully traverses the rejection. The advantages to Applicant's two-step invention have been discussed previously, and need not be repeated here.

In the 1997 Altamirano Article, in both the "batchwise" and "column chromatography" experiments, the purported osmolyte urea (2M KCl and 2M urea) was present to regenerate the mini-chaperone system. The denatured protein (cyclophilin A in 8M urea as a denaturing agent) was added to the suspension containing refolding buffer (0.1 M potassium phosphate at pH 7.8 and 5 mM 2-mercaptoethanol, a reducing agent). As such, the chaperonin-protein complex is not formed prior to the addition of the osmolyte as required by the claims. In other words, the there is no teaching of suggestion of "adding an osmolyte to the chaperonin-polypeptide complex,

thereby promoting the folding of said polypeptide from its unfolded state to its folded state to yield a folded biologically active polypeptide." Because this limitation is not taught or suggested by the 1997 Altamirano Article, Applicant respectfully requests that the Examiner withdraw the rejection under Section 102.

In paragraph 6 of the Office Action, the Examiner states that "(c) the column is then washed with a refolding solution containing the osmolyte urea at a concentration of 2M . . . ." (emphasis added). Importantly, this refolding buffer included 2M KCl and 2M urea to "develop" or wash the column prior to reuse and then "regenerate" the column after the protein to be folded had passed through the column. Thus, at no time was an osmolyte "added" to the mini-chaperonin-polypeptide complex in order to promote folding of the polypeptide. This is also true with respect to the "batchwise renaturation" experiment of the 1997 Atamirano Article as well. In such a case, the "supernatant" containing the protein to be folded is collected, and the refolding buffer is used to merely "regenerate" the gel.

Likewise, in the 1999 Altamirano Article, the refolding buffer (containing potassium phosphate and arginine) is first mixed with the refolding gel (containing either (1) the binary matrix of DsbA and minichaperone or (2) the ternary matrix of DsbA, PPI, and minichaperone). The denatured scorpion toxin Cn5 is then added to that refolding mixture. See Fig. 2 (caption); Table 1 (caption). Again, the chaperonin-protein complex is not first formed prior to the addition of the osmolyte as required by the claims.

In paragraph 7 of the Office Action, the Examiner states that "(c) the column is then washed with a refolding solution containing the osmolyte arginine and the redox agent GSH . . ." (emphasis added). Again, this refolding buffer was mixed with the protein toxin prior to addition of the chaperonin. See Table 1 caption. In addition, the buffer was used to "wash" the gel pellet

after the supernatant containing the protein to be folded was removed. See Figure 2. Again, at no time was an osmolyte added to a chaperonin-polypeptide complex in order to promote folding of the polypeptide. Because this limitation is not taught or suggested by the 1999 Altamirano Article, Applicant respectfully requests that the Examiner withdraw the rejection under Section 102.

B. Claim Rejections Under Section 103

In paragraph 8 of the Office Action, the Examiner rejected Claims 1-3, 12-19, and 40-50 as being obvious under 35 U.S.C. § 103 by based on 1999 Altamirano Article in view of Weber *et al.* The Examiner states that the 1999 Altamirano Articles does not teach the use of oligomeric chaperonins, but that Weber *et al.* teaches that oligomeric structures of GroEl/GroES are required for biologically significant chaperonin function in protein folding.

As discussed above, Applicant submits that the primary reference – the 1999 Altamirano Article – does not teach or suggest the claimed invention insofar as there is not an "addition" of an osmolyte to an already-formed chaperonin-polypeptide complex. Further, Applicant respectfully submits that the prior art actually teaches away from such a combination. More specifically, if the complete oligomeric chaperonin protein complex was used in the column chromatography device of the 1999 Altamirano Article, the binding of the polypeptide to be folded to the oligomeric chaperonin protein would be so great that the protein to be folded would not come off the column. Thus, Applicant respectfully submits that the claimed invention is non-obvious in view of the cited references. For this additional reason, Applicant respectfully submits that the Examiner withdraw the rejection under Section 103.

In view of the foregoing amendments and remarks, it is respectfully submitted that the claims are now in condition for allowance and eventual issuance. Such action is respectfully

requested. Should the Examiner have any further questions or comments in order to obtain allowance, he is invited to contact the undersigned attorney at the number listed below.

Acknowledgment of receipt is respectfully requested.

Respectfully submitted,

By: _____

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